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EXTRACTION AND ANALYSIS OF V-TYPE AGENTS (VX, RVX, CVX, AND VM) FROM VARIOUS FOOD MATRICES BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

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14. ABSTRACT:
Liquid chromatography–electrospray ionization mass spectrometry with positive-ion modes of operation was used to analyze several V-type chemical warfare agents (VX, RVX, CVX, and VM) in various food matrices. The development of a solid-phase extraction method using normal-phase silica gel columns for the extraction of V-type agents in food is described. In support of this objective, we examined select food samples using individual agents; mixtures of agents have not been studied. Various agent quantities, ranging from 1.7 to 3.1 mg, were spiked into food samples. The Agent Chemistry Branch at the U.S. Army Edgewood Chemical Biological Center has developed three analytical techniques for use, depending on the matrix. These matrices include orange juice, apple juice, whole milk, 2% reduced fat milk, Egg Beaters egg whites, tomato sauce, and several meats, including hamburger meat (80% lean and 20% fat), hot dogs, chicken nuggets, and turkey deli meat (99% fat free). The total percent recoveries (and percent relative standard deviations) for VX, RVX, CVX, and VM in various food samples are reported.

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Food	Ultra-performance liquid chromatography–time-of-flight mass spectrometry (UPLC–TOF-MS)

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PREFACE

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EXTRACTION AND ANALYSIS OF V-TYPE AGENTS (VX, RVX, CVX, AND VM) FROM VARIOUS FOOD MATRICES BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

1. INTRODUCTION

The continued threat from traditional chemical warfare agents (CWAs) such as VX (Figure 1) is evident on an almost daily basis, as current events in Syria have demonstrated. Issues ranging from food and environmental safety to compliance with treaties makes the need for low-level detection of VX of great importance. The mere existence of these molecules in either the environment or the food supply could indicate a compliance breach, even if the CWA levels were not high enough to cause any real personal harm.

Although the detection of VX metabolites and adducts from food and biological sample matrices has been reported,[1–9] limited literature exists regarding direct detection of the actual CWAs in food.[10,11] The pesticide literature often includes sample-preparation techniques that are commercially available and affordable, such as solid-phase extraction cartridges[12–20] or QuEChERS systems (Quick, Easy, Cheap, Effective, Rugged, and Safe);[21–29] however, the CWA literature seems to focus more on new techniques and specialized equipment that may not be as readily accessible to every laboratory.[9,30–35]

This document reports the efforts of the Agent Chemistry Team from the Research and Technology Directorate of the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) in developing new extraction and analytical detection methodologies using liquid chromatography–mass spectrometry (LC–MS). The objective of this task was to provide development and laboratory support for extraction of V-type agents (Figure 1) from various food samples. This includes detection and quantitative and qualitative analysis of complex matrices such as foods with high salt and fat contents. In support of this objective, we examined 10 food samples using individual agents. Mixtures of agents have not been studied. Apple juice, orange juice, whole milk, 2% reduced fat milk, Egg Beaters processed egg whites, tomato sauce, precooked turkey deli meat (99% fat free), chicken nuggets, hot dogs, and 80/20 hamburger meat (80% lean and 20% fat) represented food types that are commonly associated with school lunch programs. The choice of food types arose from collaborations and conversations with U.S. Department of Agriculture personnel. Foods were tested using commercially available normal-phase separation columns.

The use of ultra-performance liquid chromatography with time-of-flight mass spectroscopy (UPLC–TOF-MS), or any comparable high-resolution LC–MS, has become more common. From an affordability standpoint, it is currently within reach for most laboratories. For this work, extracted agent was analyzed using UPLC–TOF-MS, and percent recovery was calculated from an external calibration curve.

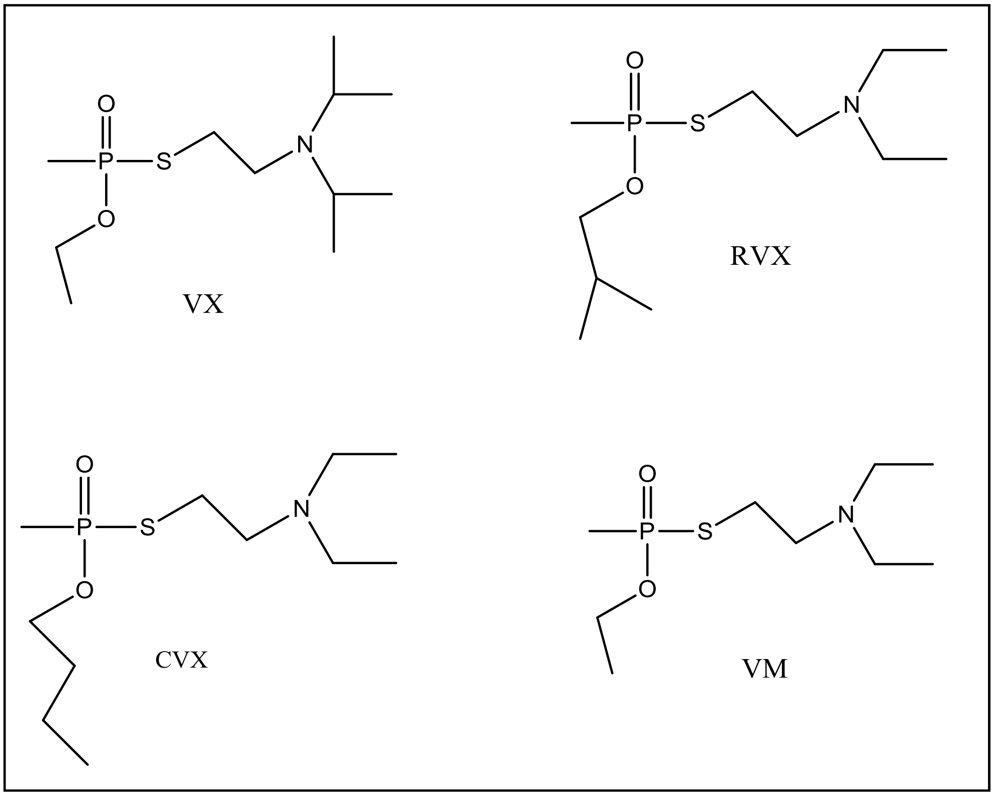

Figure 1. Structure of nerve agents VX, RVX, CVX, and VM.

2. EXPERIMENTAL METHODS

2.1 Reagents and Chemicals

The following nerve agents were provided by ECBC:

- VX: *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate;
- CVX: *O*-butyl *S*-[2-(diethylamino)ethyl] methylphosphonothioate;
- RVX: *S*-[2-(diethylamino)ethyl] *O*-isobutyl methylphosphonothioate; and
- VM: *O*-ethyl *S*-(2-diethylaminoethyl) methylphosphonothioate.

For all nerve agents, purity was >99%. All reagents and solvents were LC–MS grade. Acetonitrile, water, and triethylamine (TEA) were purchased from Sigma-Aldrich (St. Louis, MO). Apple juice, orange juice, whole milk, 2% reduced fat milk, Egg Beaters egg whites, tomato sauce, and hot dog food samples were purchased from a local grocery store (Food Lion; Edgewood, MD).

2.2 Instrumentation

All samples were characterized using an Acquity UPLC Synapt G2-S system (Waters Corp.; Milford, MA) equipped with an electrospray ionization (ESI) interface. The sampling cone voltage was 20 V. The source and desolvation temperatures were 120 and 500 °C,

respectively, and the nitrogen desolvation gas flow rate was 800 L/h. The LC–ESI–TOF–multiple reaction monitoring and LC–ESI–TOF–MS data were acquired in positive-ion scan mode over a mass range of 50–1200 Da. The leucine–enkephalin solution (1 ng/µL) was used as reference mass with a flow rate of 10 µL/min. The LC separations for all extracted samples were performed on a Waters Acquity UPLC BEH amide column (50 × 2.1 mm, 1.7 µm). The mobile phase consisted of 10 mM ammonium acetate and 0.04% NH_4OH in 90/10 (v/v %) H_2O/CH_3CN (mobile phase A) and 100% acetonitrile (mobile phase B) with a 10 µL injection volume. Separation was achieved using an isocratic condition of 20/80 (v/v %) A/B with a flow rate of 0.5 mL/min. A thermostatted column-manager compartment was used to maintain the column temperature at 35 °C and the test samples at 5 °C.

2.3 Sample Preparation and Extraction Procedures

2.3.1 Group 1: Apple Juice, Orange Juice, Whole Milk, and 2% Reduced Fat Milk

Juicy Juice apple juice (Nestlé USA; Glendale, CA), Minute Maid orange juice (Coca Cola Company; Atlanta, GA), and Food Lion brand whole milk and 2% reduced fat milk were purchased from the Edgewood Food Lion supermarket. The pH of each sample was measured and recorded before the sample was extracted. Each 2 mL sample was spiked with approximately 1.8–2.0 mg of V-type agent. The sample was diluted with 20 mL of CH_3CN. The food sample was then passed through a RediSep Rf column (Teledyne Isco; Lincoln, NE), and the eluents were collected.

2.3.2 Group 2: Egg Whites and Tomato Sauce

Egg Beaters Original egg whites (ConAgra Foods; Omaha, NE) and Ragú tomato sauce (Old World Style Ragú flavored with meat; R&B Foods; Mount Prospect, IL) were purchased from the Edgewood Food Lion supermarket. The pH of each sample was measured and recorded before the sample was extracted. Approximately 5 g of each food sample was spiked with the desired V-type agent and diluted with 10 mL of CH_3CN. The mixture was centrifuged for 15 min at 6000 rpm, and the supernatant was decanted. A second portion of 10 mL of CH_3CN was added, and the mixture was vortexed or sonicated for 1 min and again centrifuged for 15 min at 6000 rpm. The supernatant was removed, and the first and second portions were combined and passed through a silica gel column. The eluent was collected for analysis.

2.3.3 Group 3: Hot Dogs, Chicken Nuggets, Turkey Deli Meat, and Ground Beef

Orioles hot dogs (Esskay; Baltimore, MD), Smart Option chicken nuggets (Food Lion private brand), Buddig Original Deli Thin turkey deli meat (Carl Buddig & Co.; Homewood, IL), and 80/20 ground beef chuck (Food Lion sourced) were purchased from the Edgewood Food Lion supermarket. Approximately 5 g of each food sample was spiked with V-type agent and diluted with 10 mL of CH_3CN. The whole sample was homogenized using a Polytron homogenizer (Kinematica; Luzern, Switzerland) at 20,000 rpm for 1–2 min. The mixture was then centrifuged for 15 min at 6000 rpm, and the supernatant was removed. A second portion of 10 mL of CH_3CN was added, and the sample was vortexed or sonicated for

1 min and centrifuged for 15 min at 6000 rpm, and the supernatant was removed. The first and second portions of supernatant were combined and passed through a silica gel column, and the eluent was collected for analysis.

2.3.4 Extraction Procedures

A packed RediSep Rf normal-phase silica gel column (shown in Figure 2) was used in this study to separate the V-type agents from the food samples. First, the RediSep Rf column was eluted with 25 mL of 0.1% TEA/CH_3CN using in-house air to pass the solution through the column. The 0.1% TEA/CH_3CN solution was collected for later use. Second, the supernatant was passed through the column, and the sample was collected from the column. Third, 1 mL of 0.1% TEA/CH_3CN solution was added to the column and pushed slightly into the silica gel until 1 mL had just cleared the top of the silica gel. This step was repeated four times. Finally, the remaining 0.1% TEA/CH_3CN solution was added to the column and passed through the bed. The final solution was diluted with mobile phase and analyzed using LC–MS.

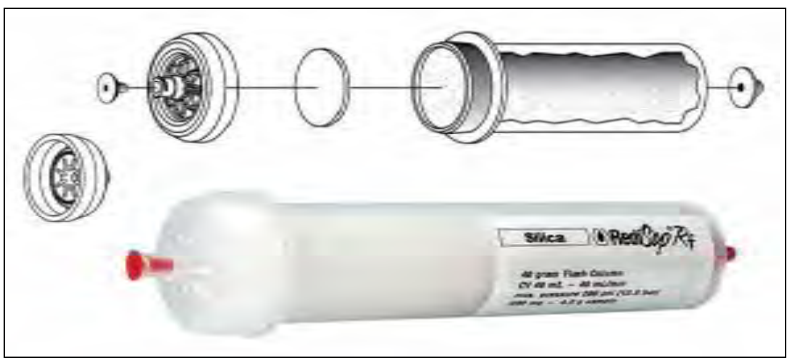

Figure 2. A RediSep Rf normal-phase silica column.

3. RESULTS AND DISCUSSION

3.1 LC Separation and Analytical Figures of Merit

For LC–MS analysis, the MS system was operated to obtain both total ion chromatograms (TICs) at *m/z* 50–1200 and extracted ion chromatograms (EICs) at *m/z* 268.1505 for VX, RVX, and CVX. EICs at *m/z* 240.1189 were obtained for VM. The TIC mode was used to ensure identification of any hydrolysis products. No hydrolysis products of V agent were found from these extracted samples. The EIC mode was used to determine the limits of detection (LODs), limits of quantitation (LOQs), and the linear dynamic ranges (LDRs) of the V-type agents. The calibration curve was plotted over concentration ranges of 0.057–960, 0.065–925, 0.126–0.700, and 0.5–0.890 ng/mL for VX, RVX, CVX, and VM, respectively, using 1 µL injections at each concentration level. The LODs for the nerve agents were calculated using 2 µL injections at concentrations as low as 0.1 ng/mL with a signal-to-noise ratio of 3:1. The LOQs for the analyte were calculated with a signal-to-noise ratio of 10:1. The linear regression equations were calculated by a least-squares analysis for the LDRs, LODs, and LOQ. The linear regression equations and the correlation coefficients are tabulated in Table 1.

Table 1. Analytical Figures of Merit Obtained for V-Type Agents[a]

Nerve Agent	LDR (ng/mL)	LOD (fg on column)	LOQ (fg on column)	Correlation Coefficient
VX	0.057–960	115	570	0.9984
RVX	0.065–925	65	130	0.9959
CVX	0.126–700	126	253	0.9956
VM	0.5–0.890	248	495	0.9953

[a]Nerve agent standard in CH_3CN.

3.2 Extraction of VX from Foodstuffs

In Figure 3b, a representative TIC is shown for each agent in various food matrices. For each agent, the EIC was obtained from the TIC of the extracted agent and is shown in Figure 3c. The mass spectrum, shown in Figure 4, exhibits mass ions at *m/z* 268.1501 due to $[M + H]^+$ and at *m/z* 128.1439 due to the loss of *O*-ethyl *S*-hydrogen methylphosphonothioate, $[M–C_3H_9O_2PS]^+$ for VX. The percent recovery calculations were based on an external calibration curve of VX (Figure 5). The results showed a >90% recovery of VX from various food matrices (Table 2).

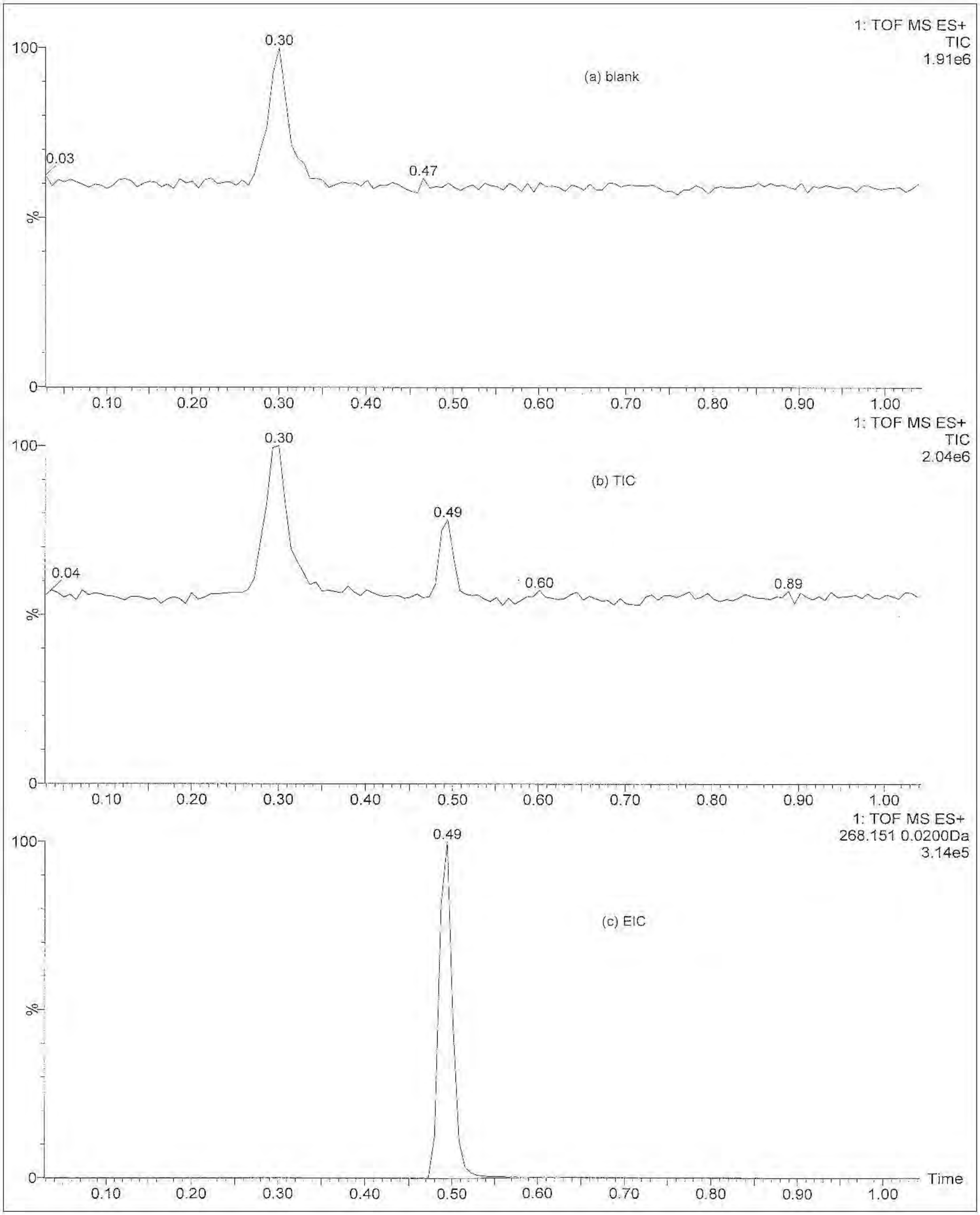

Figure 3. A representative TIC and EIC for VX extracted from apple juice:
(a) sample blank, (b) TIC, and (c) EIC.

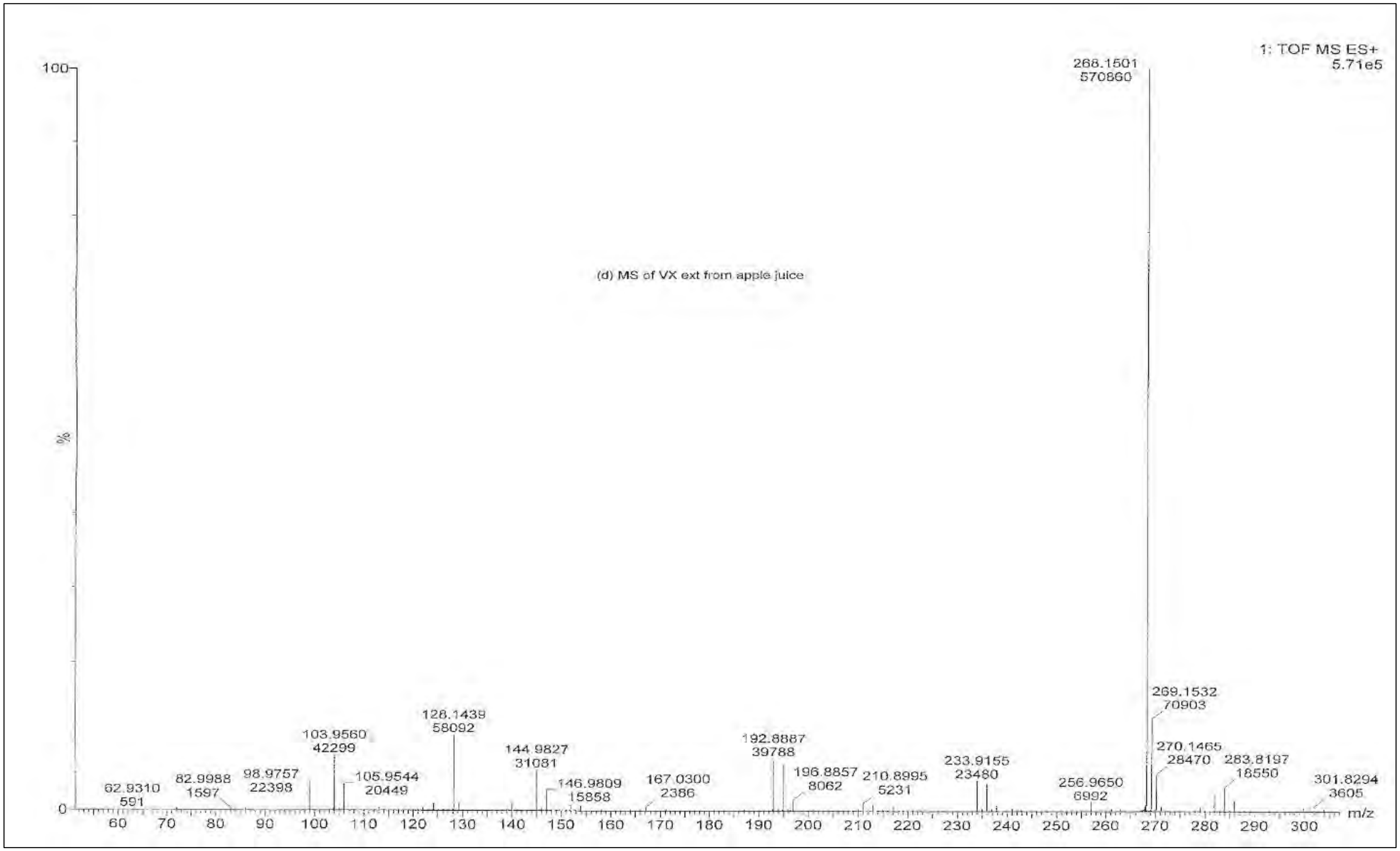

Figure 4. A representative mass spectrum for VX extracted from apple juice.

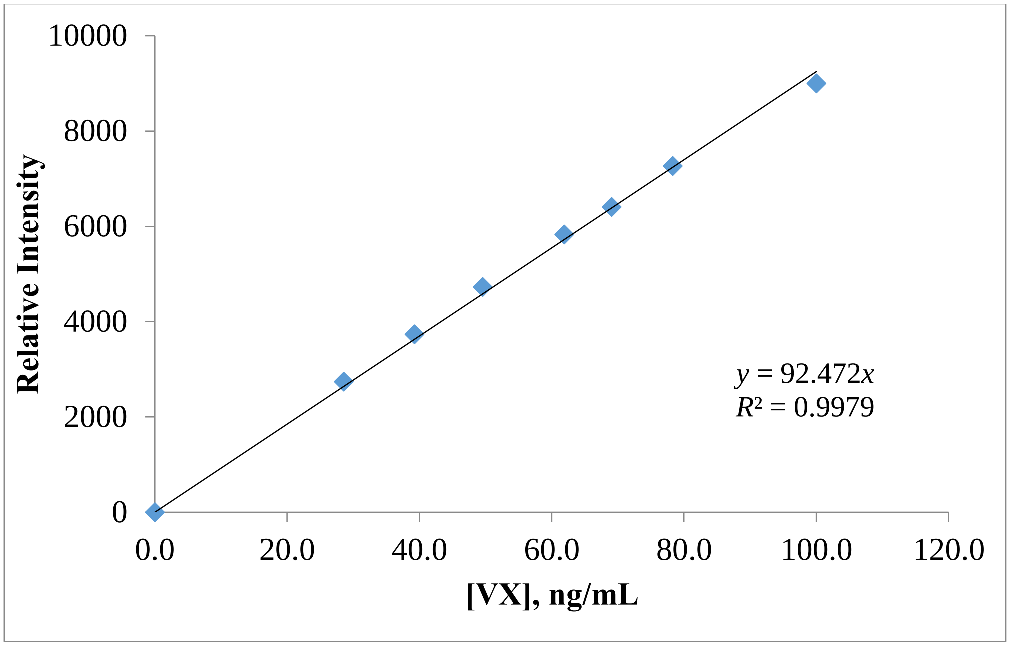

Figure 5. External calibration curve for VX in CH_3CN.

Table 2. Percent Recoveries of Extracted VX from Various Food Matrices

Food Matrix	% Recovered ± % RSD
Apple juice	94.2 ± 1.17
Orange juice	96.7 ± 2.18
Reduced fat (2%) milk	93.8 ± 2.88
Whole milk	97.4 ± 2.78
Egg Beaters egg whites	96.8 ± 1.07
Tomato sauce	97.1 ± 2.40
Turkey deli meat	93.8 ± 2.27
Hot dogs	92.4 ± 1.86
Chicken nuggets	97.8 ± 2.02
Ground beef (80/20)	96.1 ± 1.91

3.3 Extraction of RVX from Foodstuffs

In Figure 6b, a representative TIC is shown for each agent in various food matrices. For each agent, the EIC was obtained from the TIC of the extracted agent and is shown in Figure 6c. The mass spectrum, shown in Figure 7, exhibits mass ions at *m/z* 268.1505 due to $[M + H]^+$ and *m/z* 100.1127 due to the loss of *O*-isobutyl *S*-hydrogen methylphosphonothioate, $[M–C_5H_{13}O_2PS]^+$ for RVX. The percent recovery calculations were based on an external calibration curve of RVX (Figure 8). The results showed a >90% recovery of RVX from various food matrices (Table 3).

Table 3. Percent Recoveries of Extracted RVX from Various Food Matrices

Food Matrix	% Recovered ± % RSD
Apple juice	93.8 ± 2.62
Orange juice	96.4 ± 1.95
Reduced fat (2%) milk	96.4 ± 1.18
Whole milk	95.4 ± 3.73
Egg Beaters egg whites	93.9 ± 1.76
Tomato sauce	94.1 ± 2.73
Turkey deli meat	96.6 ± 2.31
Hot dogs	93.4 ± 3.51
Chicken nuggets	94.7 ± 2.95
Ground beef (80/20)	96.0 ± 2.18

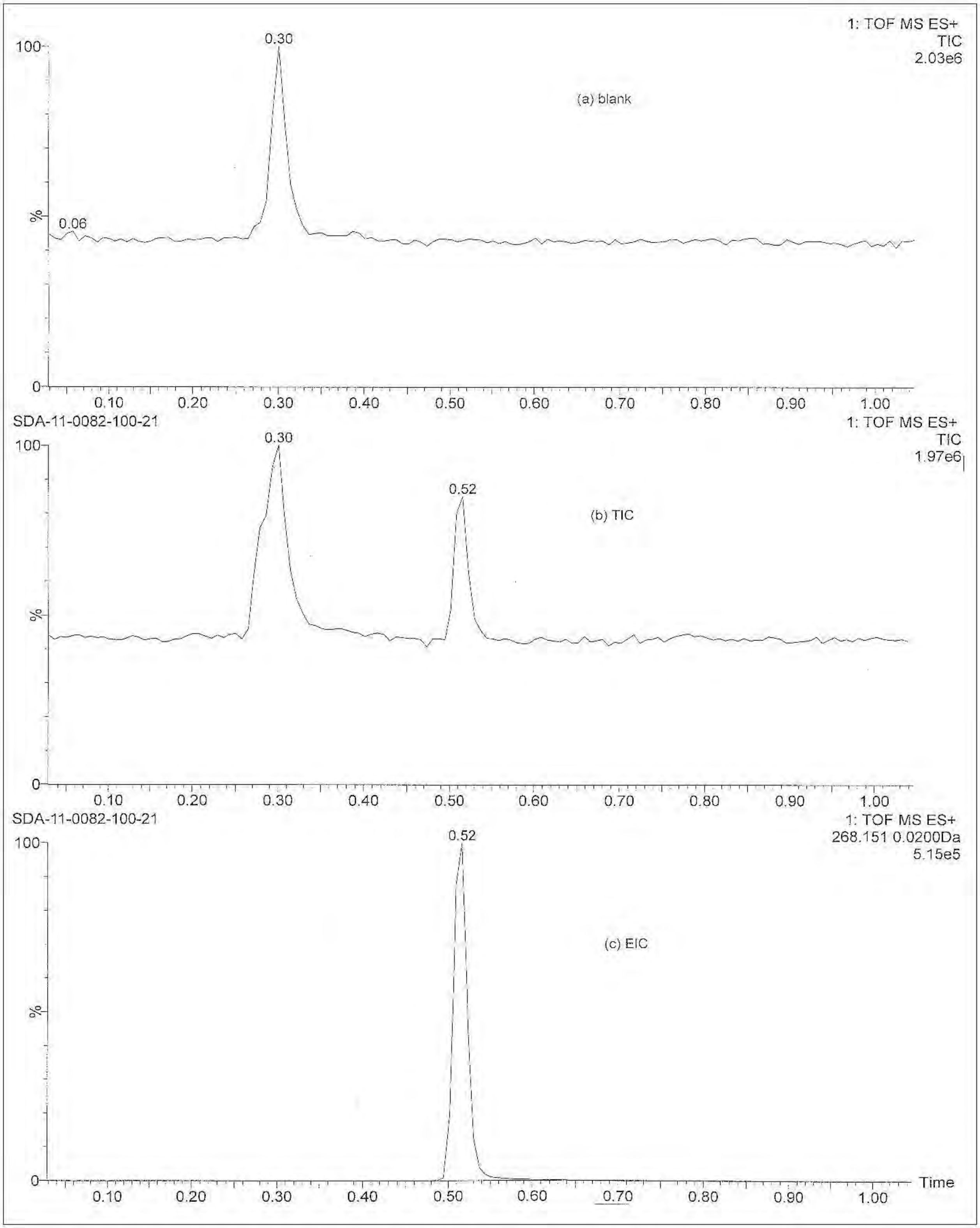

Figure 6. A representative TIC and EIC for RVX extracted from apple juice: (a) sample blank, (b) TIC, and (c) EIC.

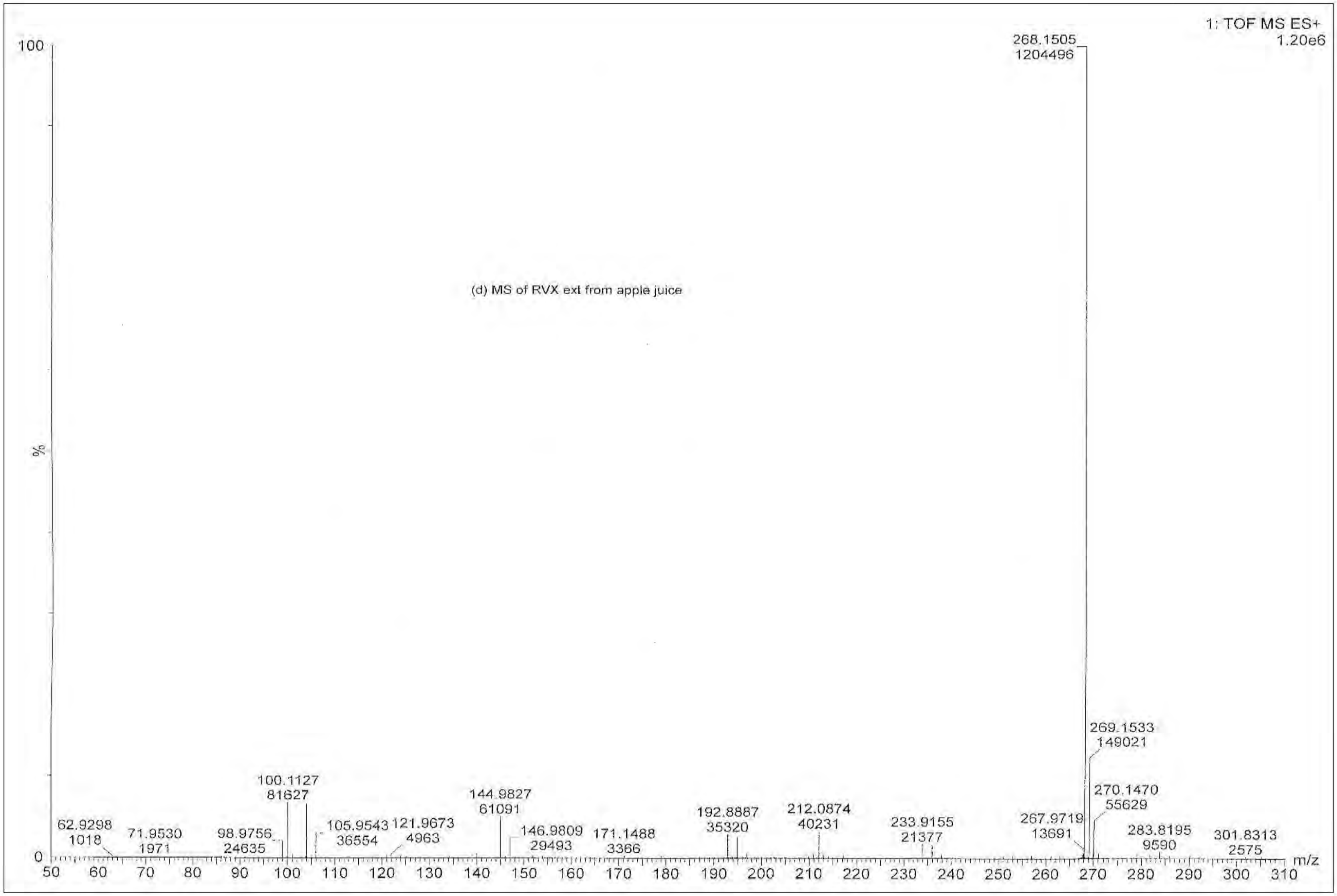

Figure 7. A representative mass spectrum for RVX extracted from apple juice.

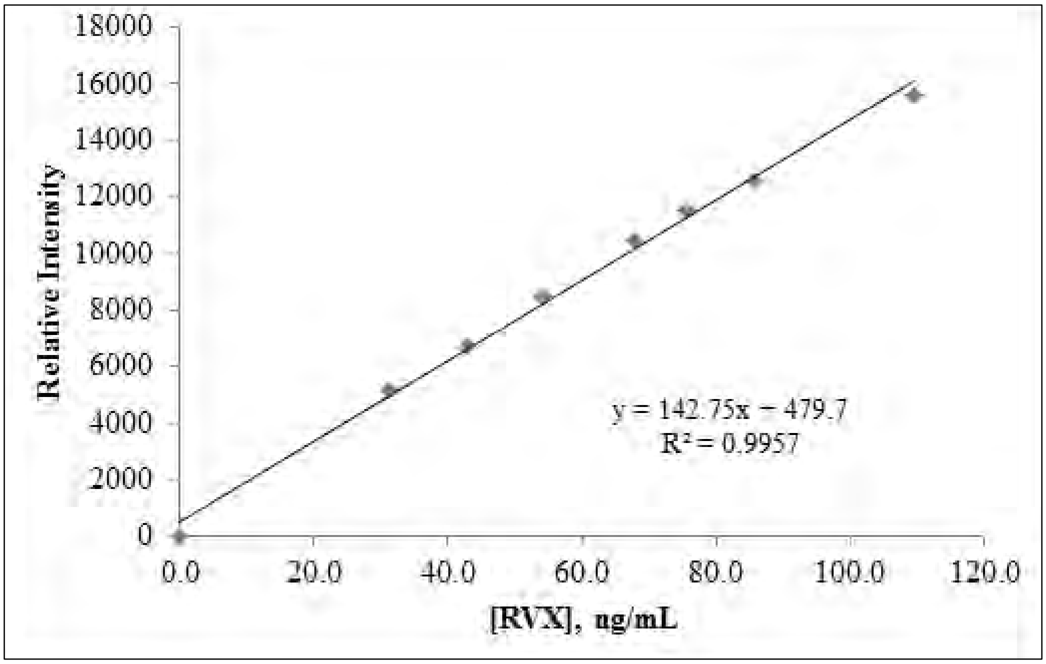

Figure 8. External calibration curve for RVX in CH_3CN.

3.4 Extraction of CVX from Foodstuffs

In Figure 9b, a representative TIC is shown for each agent in various food matrices. The EIC for each agent was extracted from the TIC and is shown in Figure 9c. The mass spectrum, shown in Figure 10, exhibits mass ions at *m/z* 268.1501 due to $[M + H]^+$ and *m/z* 100.1124 due to the loss of *O*-isobutyl *S*-hydrogen methylphosphonothioate, $[M–C_5H_{13}O_2PS]^+$ for CVX. The percent recovery calculations were based on an external calibration curve of CVX (Figure 11). The results showed a >90% recovery of CVX from various food matrices (Table 4).

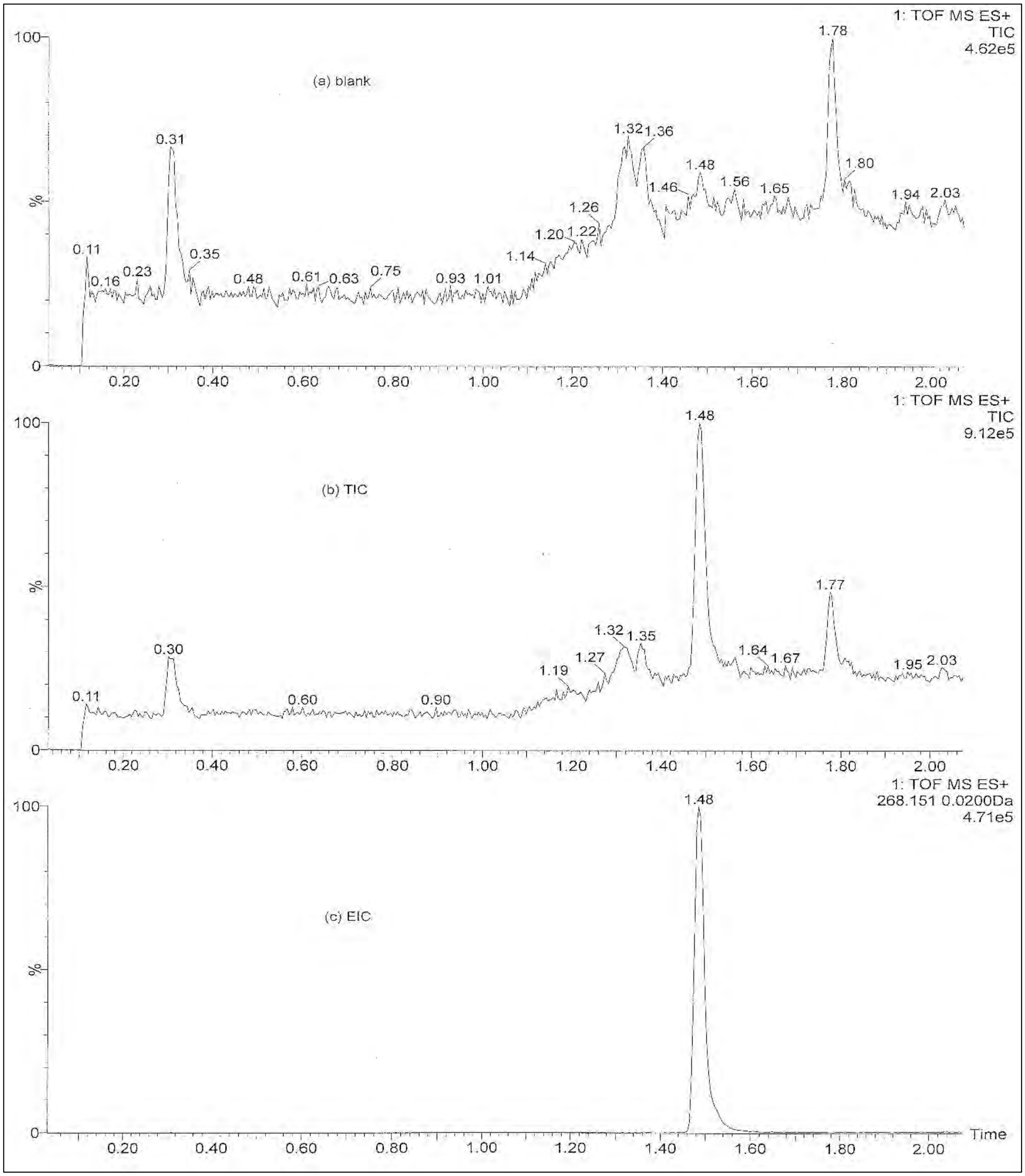

Figure 9. A representative TIC and EIC for CVX extracted from apple juice:
(a) matrix blank, (b) TIC, and (c) EIC.

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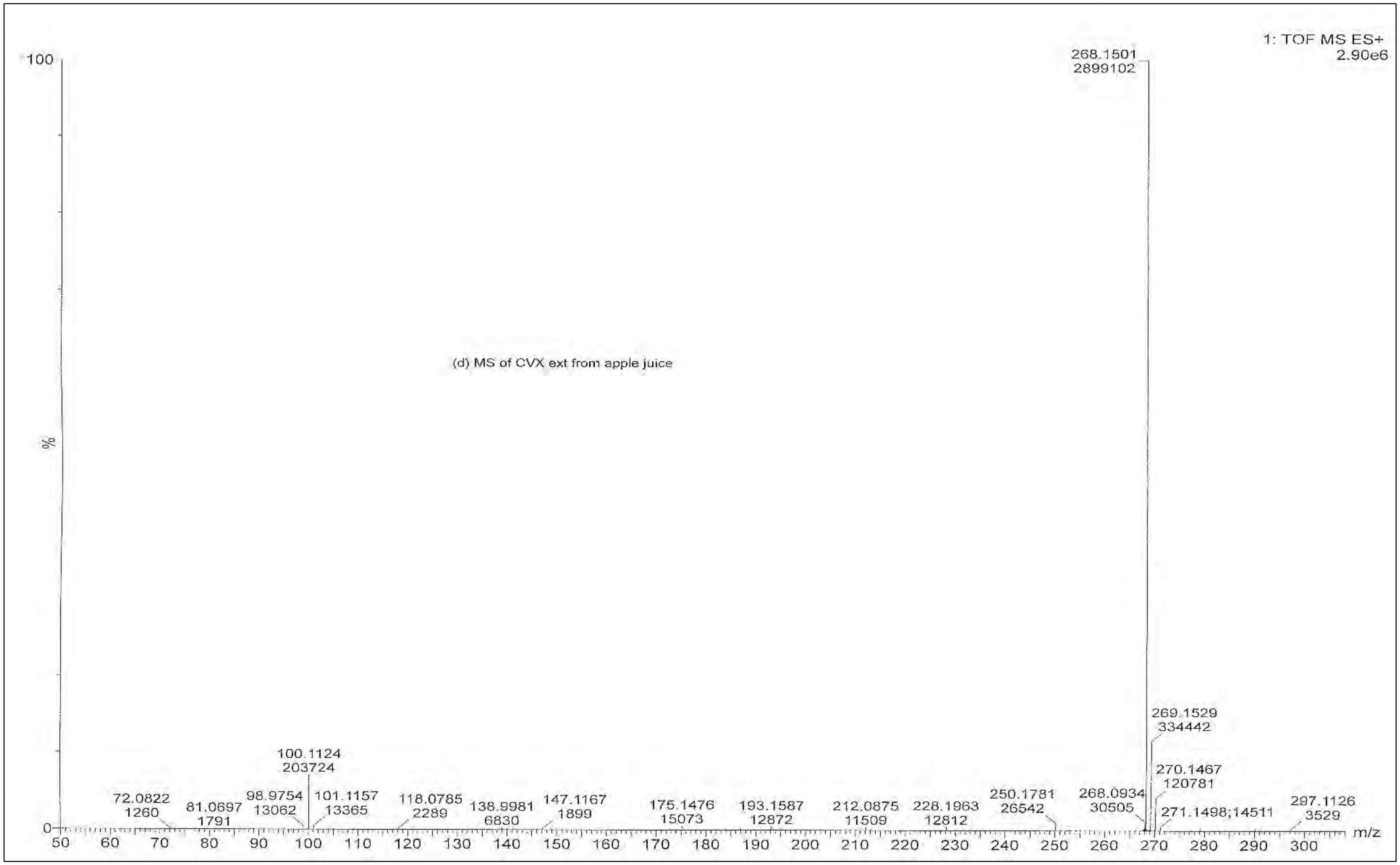

Figure 10. A representative mass spectrum for CVX extracted from apple juice.

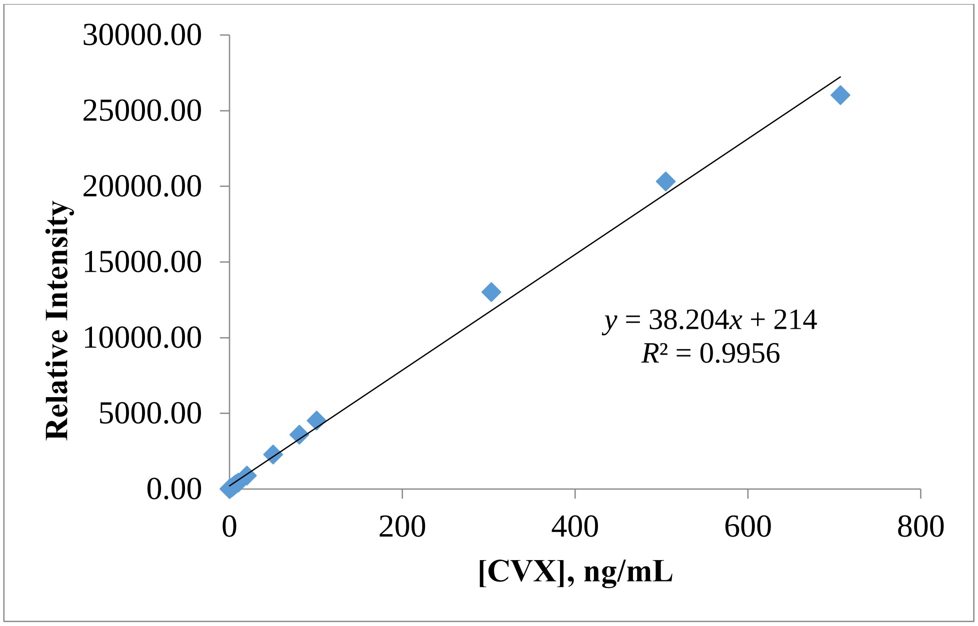

Figure 11. External calibration curve for CVX in CH_3CN.

Table 4. Percent Recoveries of Extracted CVX from Various Food Matrices

Food Matrix	% Recovered ± % RSD
Apple juice	97.8 ± 1.76
Orange juice	97.6 ± 1.58
Reduced fat (2%) milk	98.6 ± 0.58
Whole milk	99.8 ± 1.61
Egg Beaters egg whites	96.9 ± 2.02
Tomato sauce	97.2 ± 2.52
Turkey deli meat	94.9 ± 2.55
Hot dogs	93.5 ± 1.81
Chicken nuggets	96.8 ± 1.57
Ground beef (80/20)	94.5 ± 1.90

3.5 Extraction of VM from Foodstuffs

In Figure 12b, a representative TIC is shown for each agent in various food matrices. For each agent, the EIC was obtained from the TIC of the extracted agent and is shown in Figure 12c. The mass spectrum, shown in Figure 13, exhibits mass ions at *m/z* 240.1187 due to $[M + H]^+$ and *m/z* 100.1124 due to the loss of *O*-ethyl *S*-hydrogen methylphosphonothioate, $[M–C_3H_9O_2PS]^+$ for VM. The percent recovery calculations were based on an external calibration curve of VM (Figure 14). The results showed a >90% recovery of VM from various food matrices (Table 5).

Table 5. Percent Recoveries of Extracted VM from Various Food Matrices

Food Matrix	% Recovered ± % RSD
Apple juice	93.7 ± 4.09
Orange juice	96.1 ± 2.30
Reduced fat (2%) milk	98.1 ± 3.04
Whole milk	99.4 ± 1.31
Egg Beaters egg whites	94.4 ± 2.82
Tomato sauce	97.5 ± 2.27
Turkey deli meat	94.5 ± 4.48
Hot dogs	91.4 ± 1.93
Chicken nuggets	95.8 ± 3.80
Ground beef (80/20)	92.2 ± 2.07

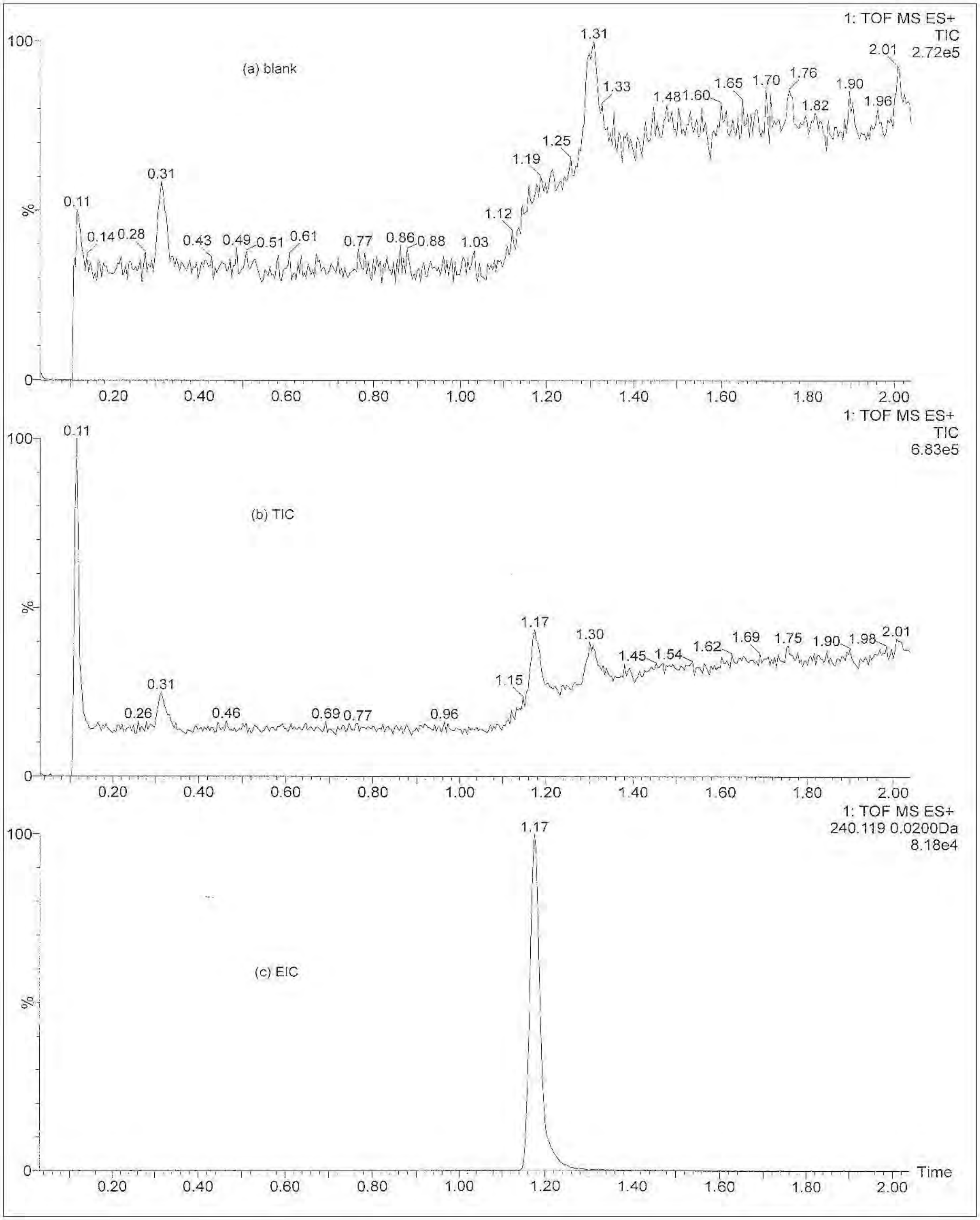

Figure 12. A representative TIC and EIC for VM extracted from apple juice:
(a) matrix blank, (b) TIC, and (c) EIC.

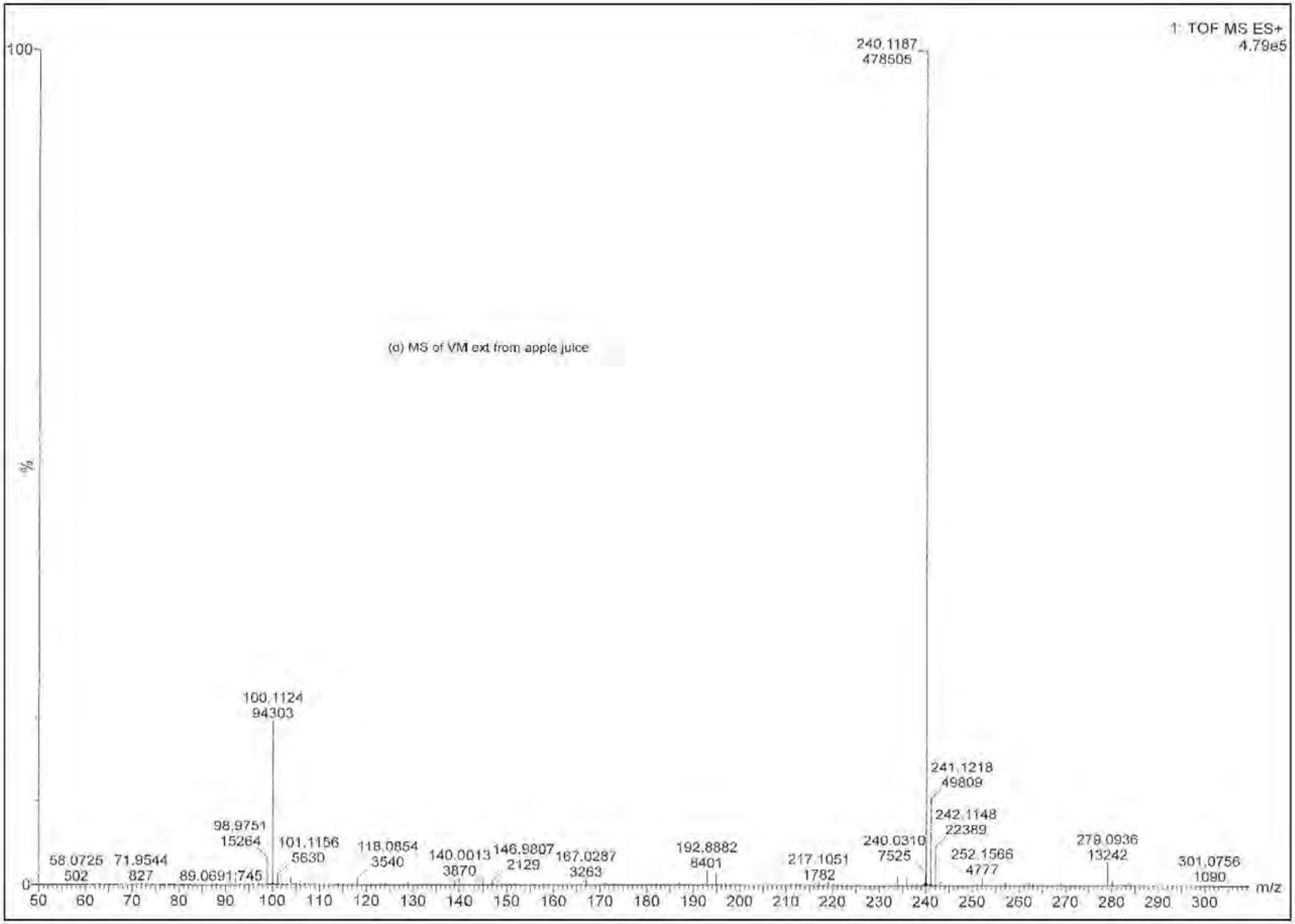

Figure 13. A representative mass spectrum for VM extracted from apple juice.

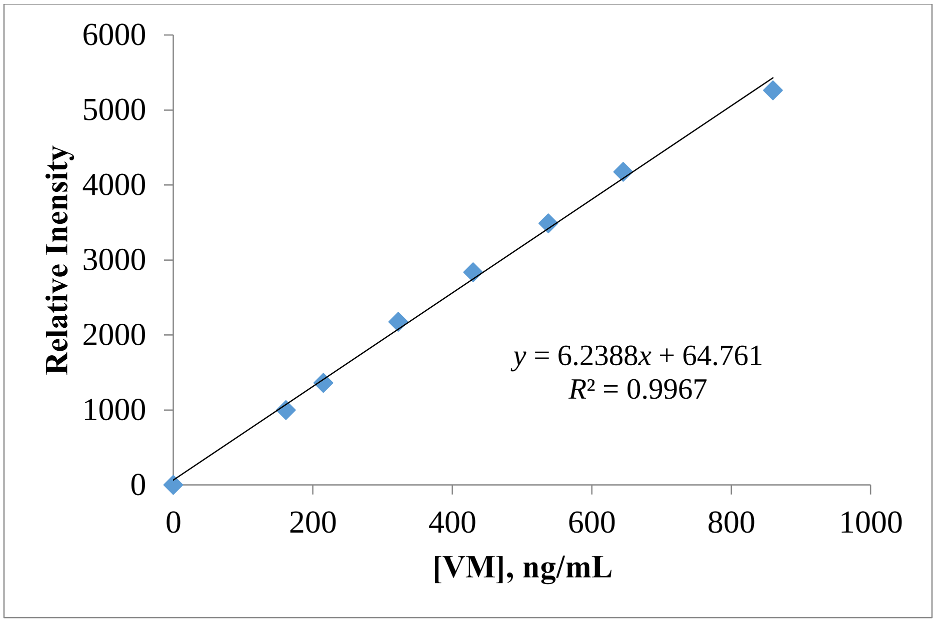

Figure 14. External calibration curve for VM in CH_3CN.

4. CONCLUSION

In this study, we set out to examine whether a commercial solution could be found for the extraction of nerve agents from various food matrices. Ideally, the method would be simple and easily available anywhere in the United States. Recoveries were >90% for the extraction techniques developed for the V-type agents in all food matrices. This report details the extraction and analysis techniques used to study V-type CWAs. The extraction method was easy to use, and it was easy to observe parts-per-million levels of VX, RVX, CVX, and VM in single food matrices.

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ACRONYMS AND ABBREVIATIONS

80/20	80% lean and 20% fat
CVX	*O*-butyl *S*-[2-(diethylamino)ethyl] methylphosphonothioate
CWA	chemical warfare agent
ECBC	U.S. Army Edgewood Chemical Biological Center
EIC	extracted ion chromatogram
ESI	electrospray ionization
LC	liquid chromatography
LDR	linear dynamic range
LOD	limit of detection
LOQ	limit of quantitation
MS	mass spectroscopy
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RVX	*S*-[2-(diethylamino)ethyl] *O*-isobutyl methylphosphonothioate
TEA	triethylamine
TIC	total ion chromatogram
TOF	time of flight
UPLC	ultra-performance liquid chromatography
VM	*O*-ethyl *S*-(2-diethylaminoethyl) methylphosphonothioate
VX	*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate

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